Growth factor and co-receptor release by structural regulation of substrate metalloprotease accessibility.

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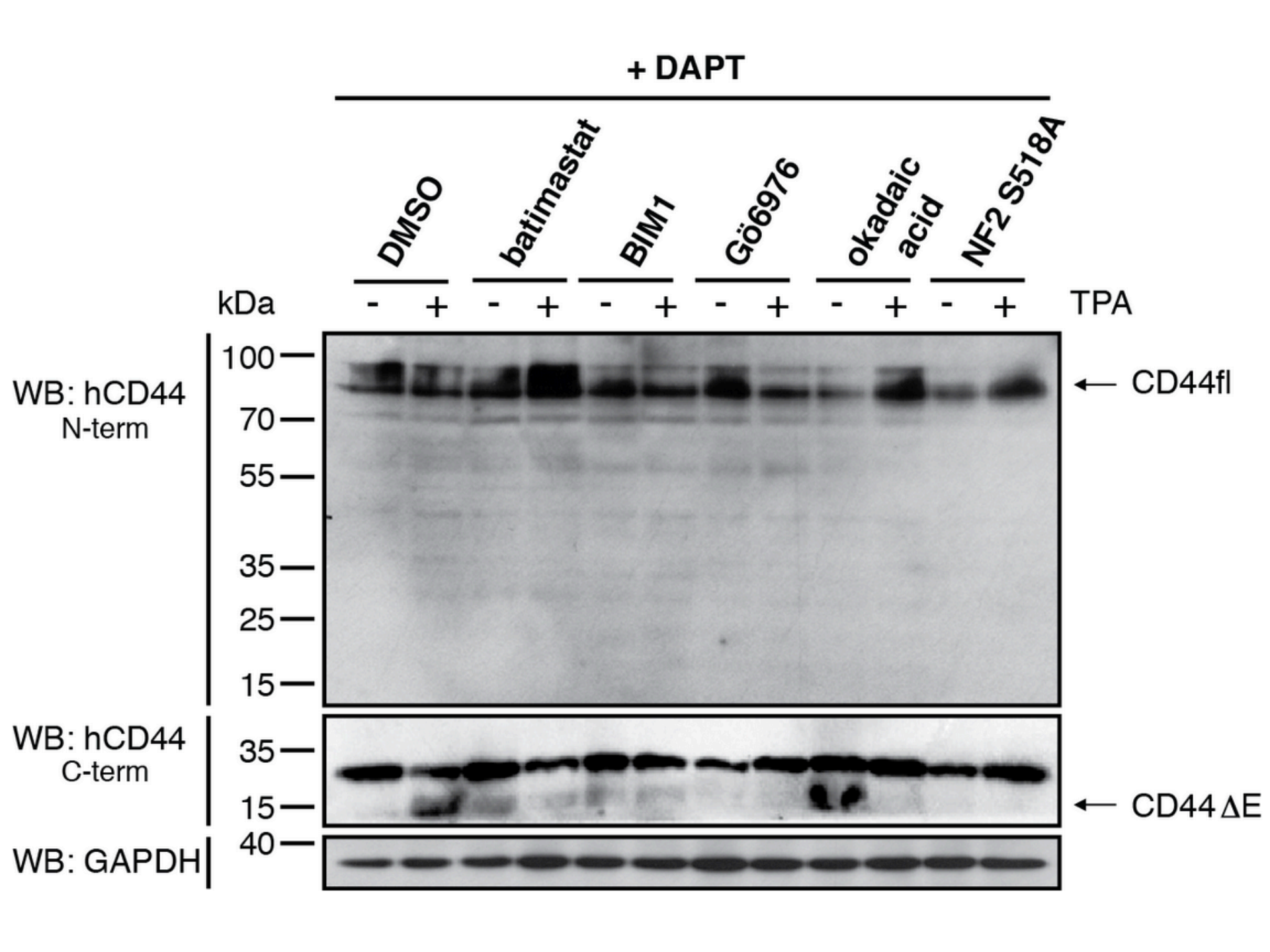
Supplemental Figure Legends:

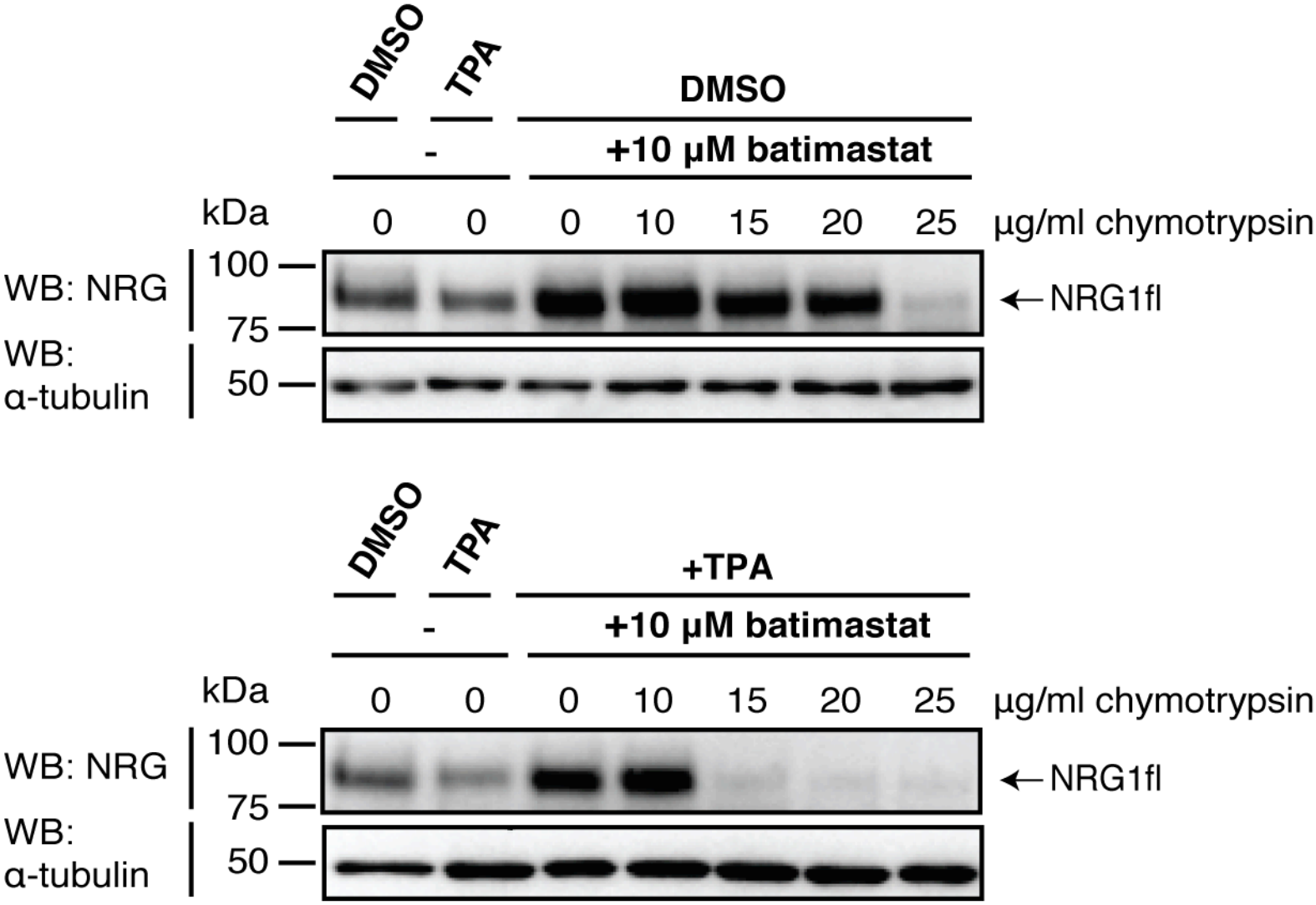
Supplemental Fig. 1: TPA induced cleavage of endogenous CD44 in the human breast cancer cell line MDA-MB-231. Cleavage is inhibited by batimastat, by the PKC inhibitors BIM1 and Gö6976, by okadaic acid and by an active mutant of merlin (see also[28,29]).

Supplemental Fig. 2: TPA induced increased chymotrypsin sensitivity of endogenous NRG1.

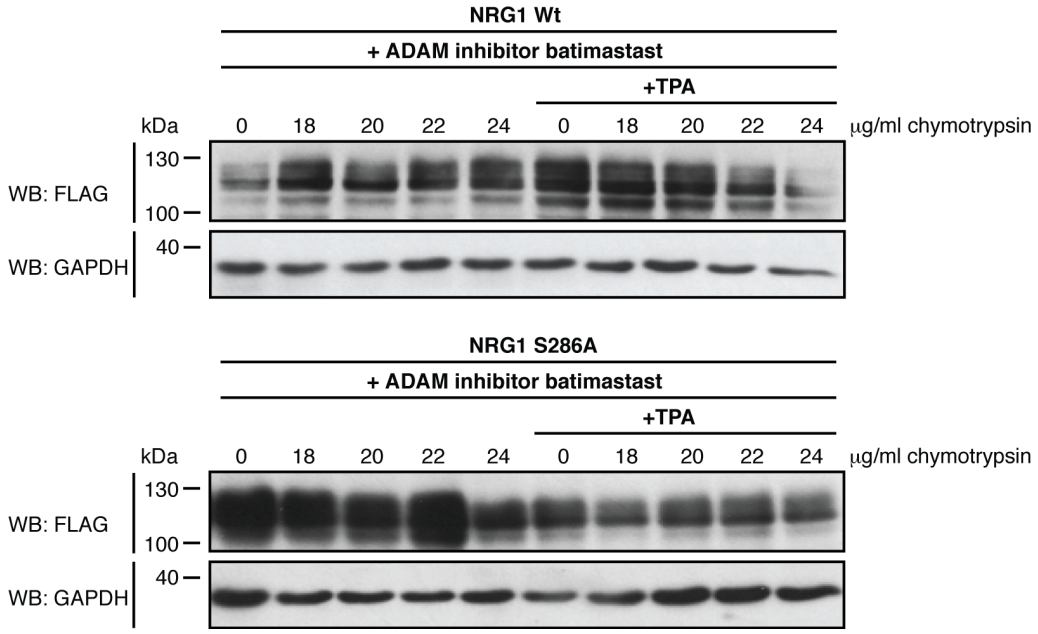
Supplemental Fig. 3: (**A**) NRG1-S286A reduces protease accessibility of the NRG1 ectodomain to chymotrypsin.

(**B**) PKCδ knockdown reduces protease accessibility of the NRG1 ectodomain to chymotrypsin.

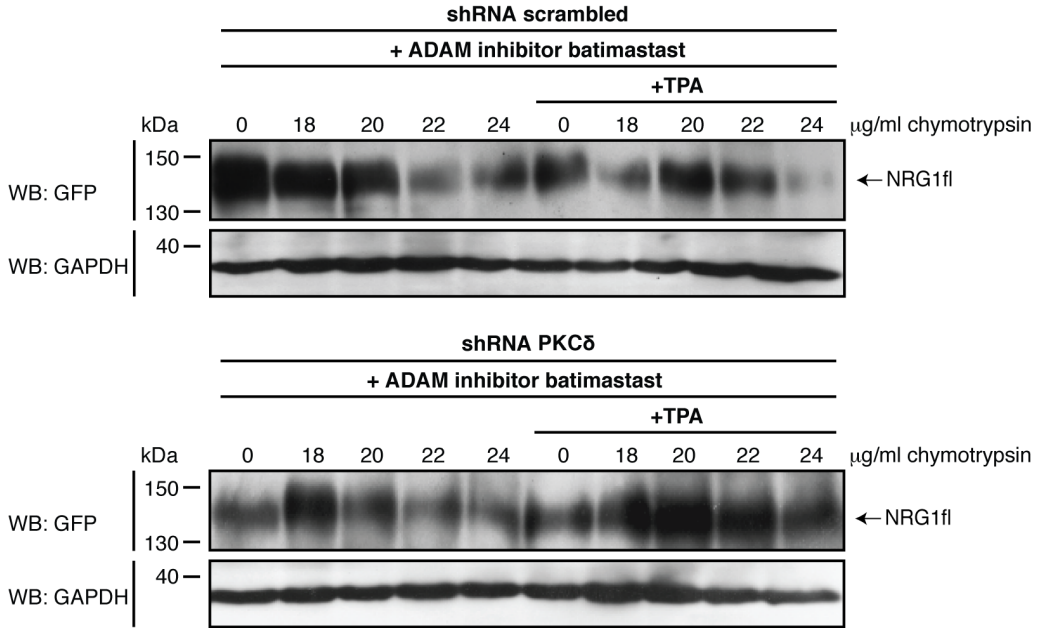

+ DAPT
DMSO
batimastat
BIM1
Gö6976
okadaic acid
NF2 S518A
kDa
- + - + - + - + - + - +
TPA
WB: hCD44
N-term
100
70
55
35
25
15
CD44fl
WB: hCD44
C-term
35
15
CD44 ΔE
WB: GAPDH
40


DMSO
TPA
DMSO
-
+10 μM batimastat
kDa
0 0 0 10 15 20 25
μg/ml chymotrypsin
WB: NRG
100
75
WB:
α-tubulin
50
NRG1fl
DMSO
TPA
+TPA
-
+10 μM batimastat
kDa
0 0 0 10 15 20 25
μg/ml chymotrypsin
WB: NRG
100
75
WB:
α-tubulin
50
NRG1fl

A


NRG1 Wt
+ ADAM inhibitor batimastast
+TPA
kDa
0 18 20 22 24 0 18 20 22 24 µg/ml chymotrypsin
WB: FLAG
130
100
WB: GAPDH
40
NRG1 S286A
+ ADAM inhibitor batimastast
+TPA
kDa
0 18 20 22 24 0 18 20 22 24 µg/ml chymotrypsin
WB: FLAG
130
100
WB: GAPDH
40

B


shRNA scrambled
+ ADAM inhibitor batimastast
+TPA
kDa
0 18 20 22 24 0 18 20 22 24 µg/ml chymotrypsin
WB: GFP
150
130
←NRG1fl
WB: GAPDH
40
shRNA PKCδ
+ ADAM inhibitor batimastast
+TPA
kDa
0 18 20 22 24 0 18 20 22 24 µg/ml chymotrypsin
WB: GFP
150
130
←NRG1fl
WB: GAPDH
40